The effect of environmental conditions on mating activity of the Buckeye butterfly, *Precis coenia*

Alice K. McDonald and H. Frederik Nijhout

Department of Zoology, Evolution, Ecology and Organismal Biology Group, Duke University, Durham, NC 27708-0325. E-mail: *hfn@acpub.duke.edu*

Abstract. The readiness of males of *Precis coenia* to court females depends on time of day, temperature, and light level. Courtship activity has a temperature optimum and, at that optimum, increasing light level dramatically enhances courtship activity. High light level appears to be critical for courtship activity, and high temperature cannot substitute for high light level. The requirement for high light intensities may be related to the behavior of males that chase females from preferred territorial perches on bright patches of exposed substrate.

Key Words: light level, temperature, courtship, *Precis coenia*

Introduction

Some years ago, when we first started to raise Buckeye butterflies (*Precis coenia* Hubner) for our experiments on the development of wing patterns (Nijhout, 1991), we encountered considerable difficulties in getting this species to mate in cages in the laboratory. Our early experimental work was done with animals produced from eggs of gravid females caught in the field. None of the standard measures to enhance mating in the laboratory such as confining the adults with various species of host plants, enlarging the mating cage, and modifying temperature and humidity, appeared to provide the necessary conditions to induce courtship and mating.

Our observations of mating activity in the field confirmed the reports by Scott (1973, 1975a,b) that courtship and mating are most frequently observed during the warmest part of the day. At these times male buckeyes perch preferentially on bright patches of sand and from those perches chase passing females. We attempted to mimic these conditions by placing our mating cages in front of a south-facing window at mid-day, and found that this immediately induced courtship behavior in males. We found that *P. coenia* would mate readily and successfully even in cages as small as 10 x 10 x 10 cm as long as they were placed in direct sunlight. It was not clear, however, whether the increased light level or the increased temperature in the mating cage was the primary stimulus for courtship and mating. Below we present an analysis of the independent effects of temperature and light level on mating activity.

Materials and Methods

Larvae of *Precis coenia* were reared in the laboratory on artificial diet and long

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day (16L: 8D with the lights-on signal at 6 am EST) conditions at 27 °C. Our laboratory colony was derived from animals collected in the Sandhills of North Carolina (Sandhills Wildlife Management Area, Richmond County). Freshly emerged adult butterflies were sexed by using characters of the prothoracic legs, and labeled on the ventral hind wing with a permanent fine tip laboratory marker. Males and females were separated and animals of each sex were grouped in separate Plexiglas cages measuring 45 x 50 x 60 cm.

Observations were made during the months of June and July 1996. For each observation session, 10 males and 5 females aged between 4 to 8 days after emergence were randomly selected from the holding cages and transferred to an observation cage (Plexiglas, 25 x 30 x 25 cm, except for some observations in the greenhouse when a wire mesh cage was used, as noted below). The data presented below are based on 6 to 10 observations sessions under each set of environmental conditions. Observation sessions were 45 minutes long. Preceding each observation session the mating cage was placed into the test environment for a 30 minute equilibration period.

Observations were made at three times of day (10 am, 12 pm, 3 pm EST), at three different temperatures, and at three different light levels. Target temperature values were 25 °C, 33 °C, and 40 °C. Due to uncontrollable drafts and imprecise temperature regulation in the greenhouse actual temperature values around these targets ranged from 23.0-28.8 °C, 30.0-38.0 °C, and 38.0–42.6 °C during the observation period. Target light level values were 2.3, 53, and 280 lux, with actual values ranging from 2.1-2.6, 49-56, and 100-430 lux around those targets due to variation in solar irradiance and physical setup of the mating cages. Precision Instruments incubators were used to provide the 33 and 40 °C temperatures under low and intermediate light intensities. Light level of 2.3 lux was provided by a 15 watt incandescent bulb, while values of 53 lux were provided by a 500 watt halogen bulb. Temperature was measured with a Yellow Springs Instrument Co., Inc. Tele-thermometer Model 46TUC. Light levels were measured with a Weston Illumination Meter, Model 756. To enable observation while maintaining the necessary temperatures, the door frame of the incubator was covered with a clear plastic sheet secured with magnets. A clear plastic tray containing one inch of a 10% $CuSO_4$ solution was placed between the cage and halogen lamp (about 6 inches from the light) as a heat absorbing filter. To achieve low and medium light intensities at 25 °C, we used the same lights in a temperature controlled room. The highest light level we used, 280 lux, is representative of the level of sunlight. It was impossible to achieve this level with artificial lights, so measures at high light intensities were made in a climate-controlled greenhouse. In the center of the greenhouse temperatures fluctuated between 32-34 °C. A wire mesh cage (45 x 45 x 45 cm) was used for observations centered around 33 °C. By placing the wire mesh cage near the cooling cells of the greenhouse, a temperature of 25 °C could be accurately maintained. To maintain 40 °C, a Plexiglas cage was placed in a sunny location; sliding vents were used to manually adjust the temperature inside the cage.

Assay of Mating Behaviors. Courtship and mating behavior in *Precis* in captivity consist of four distinctive behaviors: nudging, chasing, head dipping, and abdomen curling. These differ slightly from the general nymphalid courtship behavior

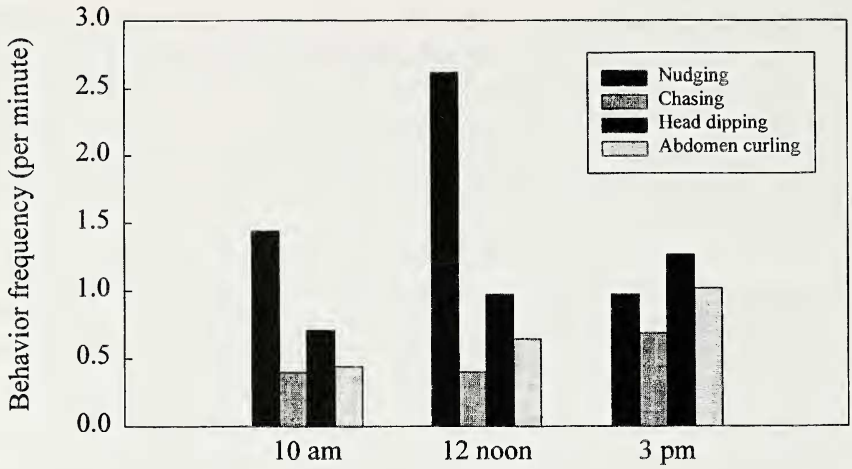

Figure 1. Mean frequency of individual courtship behaviors at each of different times of day, at the intermediate temperature of 33 °C and the high light level of 280 lux.

patterns described by Scott (1975b). Nudging is defined as the brushing of the legs of a hovering male butterfly against the wings of a perched butterfly. Often, a nudging male will land on or next to the perched butterfly. Males do not appear to distinguish between males or females at this stage in the courtship. Chasing is the pursuit, on foot, of another butterfly. Head dipping is defined as the dipping of the head under the abdomen or wing area surrounding the abdomen of the approached butterfly. This seems to serve to raise the female's abdomen into a position that the male can easily couple with. Abdomen curling consists of lateral curling of the male's abdomen towards another butterfly in an attempt to copulate. The frequencies of each of these behaviors during each 45 min observation period was scored in order to obtain a quantitative estimate of the effects of the three environmental variables on mating activity.

Results

Mating activity consistently reached a maximum at intermediate temperatures and high light intensities. Frequencies of individual mating behaviors under these conditions are shown in Figure 1. The proportion of different acts was not significantly different at 10 am and 12 noon (Chi-square, P>0.95), while the proportions of the different acts at 3 pm differed significantly from those at the two earlier times (Chi-square, P<0.0003). This difference appears to be accounted for entirely by a decrease in the frequency of nudging. At 3 pm the nudging events were significantly less frequent per unit time than they were earlier in the day (t-test, P<0.0033), whereas the frequencies of the intermediate and later courtship events did not differ significantly from their frequencies earlier in the day. This observation suggests that in the afternoon courtship becomes more efficient in the sense

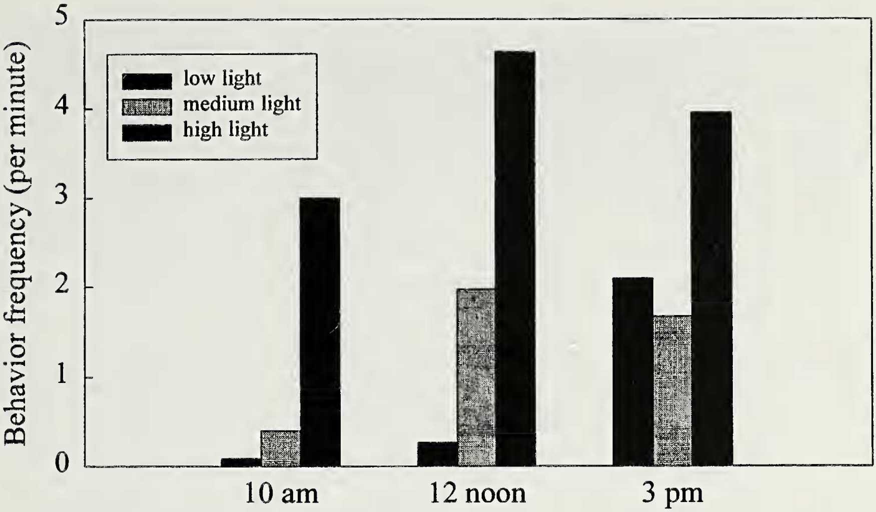

Figure 2. Mean frequency of total courtship activity at three different times of day and three different light intesities (all data shown is at 25 °C).

that a larger proportion of individuals that begin courtship are able to take it to completion.

High light intensity dramatically increased mating activity at all times of day (Figure 2). At 10 am and 12 noon a stepwise increase in light intensity significantly increased mating activity ($P<0.003$ for all pairwise comparisons). At 3 pm mating activity at low and medium light intensities were not significantly different from each other but mating activity increased significantly at the highest light intensity ($P<0.0004$).

The Combined Response to Temperature and Light Level. The overall mating activity was assessed as the sum of all observed courtship events. These are graphed in Figure 3 as a function of both temperature and light level at three different times of day. Contours were calculated using SigmaPlot (Jandel Co.). In view of the fact that the numerical tally of mating activity is dominated by early courtship events (nudging, see Figure 1), the overall mating activity was also estimated based on the intermediate and late courtship events alone (Figure 4). Both measures of mating activity revealed a qualitatively similar pattern. At 10 am, mating activity increased when temperature and light level increased. The effect of increasing temperature was more pronounced at high light intensities than at low light intensities, while the effect of light level was most pronounced at intermediate to high temperatures (Figures 3a, 4a). At 12 pm and 3 pm, by contrast, there was a distinct optimal temperature for mating activity (32–34 °C; Figures 3b,c, 4b,c). At 12 pm, increasing light level had a stimulatory effect on mating activity at optimal temperatures and below, whereas at temperatures above the optimum, light level had little effect on mating activity (Figure 4b). At 3 pm, light level affected mating behavior only within the

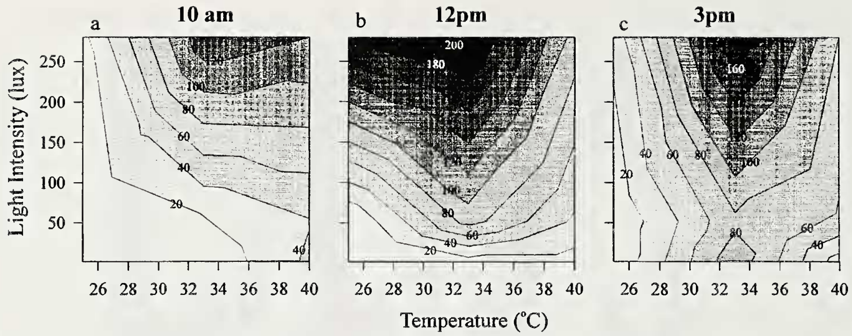

Figure 3. Mating activity at different times of day as a function of both temperature and light level. Mating activity (numbers on contours) was scored as the sum of the frequencies of all 4 of the courtship behaviors (Fig. 1) during a 45 min observation period.

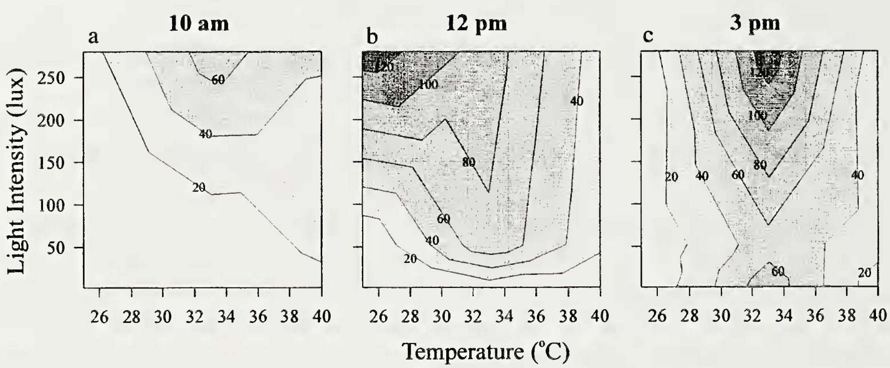

Figure 4. Mating activity at different times of day as a function of both temperature and light level. Mating activity (numbers on contours) was scored as the sum of the frequencies of the three late stages in courtship (chasing, head dipping, abdomen curling) during a 45 min observation period.

optimal temperature range, whereas light level had little or no effect on mating activity at temperatures below or above the optimum (Figure 4c).

Analysis of significance of pairwise comparisons of the data presented in Figures 3 and 4 revealed the following. In Figure 3, pairs of points that differ by more than 55 events (approximately 3 contour intervals) are significantly different from each other (t-test, $P<0.05$), both within and between panels. In Figure 4, pairs of points that differ by more than 26 events (slightly greater than 1 contour interval) are significantly different from each other (t-test, $P<0.05$), both within and between panels.

Discussion

The mechanisms that regulate mating behavior result in dramatically different responses to temperature and light level as time of day progresses.

Maximum mating activity always coincided with temperatures of 32–34 °C and high light level (280 lux), regardless of time of day. The relative frequency of nudging, the earliest event in courtship behavior, peaked at 12 pm, while the relative frequency of abdomen curling, the final event preceding copulation, was greatest at 3 pm. Therefore, although total courtship activity at noon was greater than at 3 pm, the final stages of courtship, and presumably mating success, were relatively more frequent at 3 pm.

Rutowski (1991) has outlined three hypotheses to explain why males court preferentially at certain times of day. First, limited thermoregulatory capacity may restrict mating activity to periods when the environmental temperatures are neither too high nor too low. Second, mating may be timed to coincide with female emergence times. Third, mating may be timed so as to minimize interference between species. Our results illuminate the first of these hypotheses, but show that mating activity is not constrained strictly by temperature. Although male *Precis coenia* clearly have an optimal mating temperature of 32–34 °C, exposure to this temperature alone did not result in maximum levels of mating activity. Mating activity at 12 pm and 3 pm was consistently higher than at 10 am. Time of day, therefore, affects mating behavior independently of temperature. Light level also has an independent effect on mating behavior. Within the optimal temperature range, high light level increases mating activity at all three times of day, whereas at non-optimal temperatures the effect of light level depended on the time of day.

Scott (1975b) has noted that in nature, mating activity of *Precis coenia* occurs mostly in late morning and early afternoon. Our results suggest that, given the right combination of temperature and light level, mating behavior can occur at most times of day, although the interaction of light level, temperature, and time of day ensure that the bulk of mating activity is most likely to occur in the early afternoon.

If light level is low, little mating activity occurs, even at optimal and higher temperatures. It is not clear at present why light level should have such a great effect on mating activity. It is possible that high light level acts indirectly, by elevating the male's body temperature. This would imply that the optimal body temperature is substantially higher than the 32–34 °C optimal environmental temperature we measured. Optimal body temperatures for flight in insects range from 35 to 42 °C (Heinrich, 1993), so it is conceivable that mating activity could also require such high body temperatures. However, if the effect of light level was mediated through an elevation of body temperature, one would expect high light intensities to be more effective at inducing mating activity at temperatures below the environmental optimum than at temperature above the optimum. The apparent temperature optima in Figures 3 and 4 would then be expected to be a function of light level, with a lower temperature optimum at high light intensities and a higher temperature optimum at low light levels. Instead, the temperature optima are unaffected by light level (except for a single instance at low temperatures: Figure 4b), suggesting that these two environmental variables do not interact, and that light level seems to be important for reasons other than radiational heating.

One explanation for the evolution of high light level as a cue for mating may be found in the fact that mating activity of the *Precis coenia* population we studied occurs preferentially in open habitats on exposed patches of sand. Such bright areas in the landscape serve as perching territories for males (Scott, 1975b, and personal observations), and from these territories males chase passing insects, including females and other males. Males are chased away, and females, if receptive, land nearby and courtship begins (Scott, 1975b). It is possible that the selection of perching territories is guided primarily by brightness of the substrate. If the acquisition of such a territory is important for mating success, then it seems reasonable to suppose that a response to high light levels as a stimulus for courtship may have evolved in association with the behavior by which males select especially bright perching territories from which to chase passing females.

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